Mapping of chromosome 3 alterations in human breast cancer using microsatellite PCR markers: correlation with clinical variables

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Abstract. Human breast tumours were analyzed with polymorphic microsatellite markers specific to chromosome 3p. Allelic imbalance (AI) was observed in 34% of the tumours. Microsatellite markers from two regions showed higher percentage of imbalance suggesting the presence of tumour suppressor genes or genes important for malignancy. Microsatellite instability was also found, implying errors in DNA replication. No significant correlation was found between AI and conventional prognostic variables. However, a striking correlation was found between AI and tumour S-phase fraction; AI was also significantly correlated with low steroid receptor content. A multivariate model including prognostic variables, showed that AI was without exception a significant prognostic variable; patients having tumours with AI had approximately a four-fold increase in relative risk of death. We conclude that screening for 3p deletions gives prognostic information and further investigations should reveal whether this finding could assist in treatment of the disease.

Introduction

Genes that play an important role in controlling cell division or differentiation and are critical in the pathogenesis of malignancy are termed oncogenes or tumour suppressor genes. The localization of these genes to specific chromosome regions is a major effort in cancer research today. Many abnormalities, e.g. deletions and translocations, in the genome of breast cancer cells have been detected and sites of susceptibility have been found on several chromosomes (1-2).

A genetic locus at 3p modulates tumorigenicity, as shown by *in vivo* studies in immunodeficient mice, indicating the presence of a putative tumour suppressor gene(s) (3,4). The 3p chromosome region is eliminated in the course of progressive tumour growth in SCID mice (5). Many studies have shown abnormalities on the short arm of chromosome 3 in carcinomas of various organs: breast (6), kidney (7), lung (8), ovary (9), cervix (10), testis (11) and nasopharyngeal carcinoma (12) giving reason to focus on 3p in the search for a candidate tumour suppressor gene or group of genes. Studies on short term cultures of breast carcinomas suggest that the loss of genetic information from 3p loci is an early and possibly primary event in tumorigenesis (13). Detection of 3p abnormalities could therefore prove valuable in determining the status of breast tumour progression.

Detection of loss of heterozygosity in tumour material with polymorphic markers for specific chromosomal loci gives information towards localizing the 3p tumour suppressor gene(s). Since microsatellite polymorphisms are highly informative, abundant and well distributed throughout the genome they are an important tool in deletion and imbalance mapping. Deletion studies in which many tumour samples with such polymorphic markers are screened could allow the localization of the tumour suppressor gene to a small region of 3p.

In this study breast tumours were screened for allelic imbalance on chromosome 3 using highly polymorphic markers focusing on the 3p14 and 3p21 sites. Furthermore, we tested whether these findings were correlated with conventional prognostic variables, steroid receptor content, DNA ploidy and S-phase fraction and patient outcome.

Materials and methods

Patients and tumour material. A fresh biopsy from primary breast tumours from patients diagnosed with breast cancer in Iceland is routinely sent to our laboratory for estrogen and progesterone receptor (ER and PgR) analysis with the dextran-coated charcoal method. Blood samples from the patients are collected in EDTA and if not processed

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Table I. Information about the markers used in this study.

Marker	Reference	Cytological location	Number of samples	het (%)	AI (%)
D3S726	24	3p21-3pter	35	19 (53)	3 (16)
D3S1211	25	3p22	68	57 (84)	19 (33)
RIK	26	3p21.3	50	29 (58)	8 (28)
PH3H2	27	3p21.3	32	13 (41)	6 (46)
D3S1029	28	3p21.2-21.3	43	35 (81)	8 (22)
D3S1076	28	3p21.1	43	32 (74)	6 (19)
D3S1067	28	3p14.3-21.1	115	78 (68)	18 (23)
D3S1233	29	3p14	96	51 (53)	17 (33)
D3S1217	25	3p14	132	98 (74)	27 (28)
D3S1210	25	3p14	131	99 (76)	26 (26)
D3S1101	30	3p12-14	90	75 (83)	12 (16)
GLUT 2	31	3q26	52	45 (86)	11 (24)

immediately the tumour tissue and blood are quick frozen at -70°C . All relevant information about the tumours, e.g. size, type, node status, was recorded by the Department of Pathology, the National University Hospital of Iceland. The ratio of tumour versus normal cells in the samples was also evaluated by histological examination. It has been suggested that loss of heterozygosity in tumours is detectable in samples with 60% contamination with normal cells (14), and work in our laboratory has shown that a 55% tumour cell fraction in a sample is sufficient for detection of allelic imbalance (Arason, unpublished results). Therefore, only tumours with a score of more than 55% (tumour cell as percentage of total cell population) were included in the study. 140 breast tumours were analyzed.

DNA extraction and analysis. Normal DNA was extracted from leukocytes (15) and tumour DNA was extracted either from the nuclear pellet after cytosol removal for hormone receptor analysis or from the pulverized primary tumour itself according to the method described by Maniatis *et al* (16). Paired blood and tumour DNA was subjected to PCR analysis. DynaZyme™ (Finnzymes Oy) polymerase was used in the buffer solution provided by the manufacturer. The markers used, listed in Table I, were obtained from Scandinavian Gene Synthesis AB, Isogen bioscience and the Nordic primer bank in Uppsala (Dr C. Wadelius, Dept. Clinical Genetics, Univ. Hospital, Uppsala) and end-labelled with [γ - ^{32}P]ATP using T4-PNK. One marker is located on 3q and was used mainly on samples which showed abnormalities on 3p to test for total chromosome 3 loss. The PCR products were subjected to denaturing gel electrophoresis and detected by autoradiography. 50 cases were analyzed by Southern blot analysis (16). The DNA probes used were RIK, PH3H2 and D3S726 (Table I).

DNA ploidy and S-phase. The DNA content in individual cell nuclei was analysed in a FACScan instrument after staining with propidium iodide (17,18). In accordance with the Convention of nomenclature for DNA cytometry (19),

tumour ploidy was defined as follows: one DNA stem cell population as diploid and two or more DNA stem cell populations as non-diploid. The percentage of nuclei corresponding to S-phase fraction was calculated with a planimetric method (20). The median S-phase (7%) fraction was used as the cut-off level for low and high rate of proliferation.

Statistical analysis. A chi-square test was used to assess the relationship between 3p imbalance and other prognostic variables. Prognostic factors were categorized using commonly applied cut-off levels (Table III). Survival curves were calculated according to the method of Kaplan and Meier (21). Tests of difference between curves were made with the log-rank test for censored survival data (22). Multivariate analyses were performed with Cox's partially non-parametric regression model (23). The Statistica/Mac package (Statsoft, Inc) was used for the statistical analyses.

Results

Allelic imbalance on 3p. The breast tumours were tested for loss of heterozygosity (LOH) or allelic imbalance (AI) using CA-repeat and restriction fragment length (RFLP) polymorphisms. 11 different markers were used to screen chromosome 3p and one marker is located on 3q. In general the CA-microsatellite markers were more informative than the RFLP probes. Fig. 1 shows an autoradiogram of selected representative cases where imbalance was found by visual observation. The autoradiogram cannot discriminate between deletions and amplifications, and allelic imbalance includes both alterations. However, as observations of amplifications in this region of the genome is not common in the literature, the imbalance observed is most likely due to deletions. 48 of the 140 (34%) samples showed AI with at least one marker. AI for individual markers ranged from 16-46% (Table I). The markers D3S726 (3p25-p26), D3S1076 (3p21.1-p21.2) and D3S1101 (3p12) gave the lowest percentage of abnormality and high percentage was detected with markers in the 3p21.3

Table II. Clinicopathological information of the 6 cases with extra alleles (RER cases).

case	no. of markers with RER/tested	ER status	PgR status	node status	S-phase %	ploidy	3pAI
567	1/19	+	+	-	8.6	diploid	+
593	4/19	+	+	+	7.9	diploid	-
983	1/17	-	-	-	40.0	non-diploid	+
990	9/29	+	+	+	5.1	non-diploid	+
993	1/30	-	-	-	13.5	non-diploid	+
1019	5/21	ND	ND	-	16.9	non-diploid	+

ND = not determined.

Figure 1. Dinucleotide repeat polymorphisms in selected matched normal (N) and tumour (T) tissue from breast cancer patients. The microsatellite markers D3S1210 and D3S1217 were used in PCR analysis and the 32 P-labelled amplification products separated in 6.5% polyacrylamide, 8 M urea denaturing gels. Case numbers are shown at the top. Numbers to the right indicate the size (in nucleotides) of the marker V (Boehringer Mannheim). AI (allelic imbalance) can be seen with D3S1217 in all heterozygote cases, except 990 (homozygotes are 415, 799 and 981). AI can be seen with D3S1210 in all heterozygote cases (homozygotes are 769, 982 and 990).

(46%) and 3p14 (33%) chromosome regions. Imbalance on 3q was observed in 11 of the 48 samples with 3p AI. Only two cases of these 11 showed imbalance with all informative markers tested, suggesting the loss of the whole chromosome 3. In the only male (not included in the statistical analysis) breast cancer sample examined a deletion was observed with one (D3S1210) of 5 informative markers tested.

Microsatellite instability. Extra bands in electrophoretic patterns (Fig. 2) were found in 6 of the 140 (4%) tumours studied. Additional markers from other chromosomes confirmed this microsatellite instability for 3 tumours which showed such instability at more than one locus. The remaining 3 were altered at only one locus. AI at 3p was detected in 5 of the 6 tumours (83%). Clinicopathological information for these 6 tumours is shown in Table II.

Figure 2. Analysis of paired normal (N) and tumour (T) DNA from patient 1019 at loci on 3p and 16 q defined by the microsatellites D3S1210 and D16S413. PCR-amplified fragments were subjected to denaturing gel electrophoresis as in Fig. 1. Fragment sizes (in nucleotides) are indicated at the side of the lanes. Extra bands in the tumour DNA can be detected as faint bands of higher molecular weight (160 and 129 nucleotides, respectively), indicated by arrows.

Allelic imbalance on 3p (3pAI) in relation to other prognostic variables (Table III). A striking correlation was found between 3pAI and tumour S-phase fraction with 76% of tumours with 3pAI having a high S-phase and 37% of those without 3pAI ($p=0.0000$). A significant correlation was also found between 3pAI and non-ploidy and steroid receptor negativity. However, there was not a significant correlation between 3pAI and conventional prognostic factors (patients' age, tumour size and axillary lymph node involvement).

Allelic imbalance on 3p (3pAI) in relation to survival. There was a significant prognostic separation of survival curves in relation to 3pAI (Fig. 3). After 5 years of follow-up there was approximately 50% mortality among patients with 3pAI and about 15% among those without 3pAI ($p=0.0000$). In node-negative patients, 3pAI was also connected with increased mortality ($p=0.0307$). Multivariate analyses were undertaken

Figure 3. Overall survival. The number of patients at risk at time 0, at 2.5 years and 5 years is shown for both categories. — patients without 3p AI; - - patients with 3pAI.

to evaluate the possible clinical relevance of 3pAI as a prognostic factor in breast cancer. In the patient series as a whole, patients having tumours with 3pAI had a four-fold increase in relative mortality rate compared with patients without 3pAI. Tumour size, axillary lymph node involvement and S-phase fraction were also of independent prognostic value (Table IV). In the node-negative setting, multivariate analysis did again show that tumor size, S-phase fraction and 3pAI had each about a four-fold increase in the relative risk of mortality. However, this did not reach statistical significance for any of the variables as only 69 patients were included in that analysis (RR of mortality was the following for each variable: tumour size 3.4, 95%CI 0.6-20.4; S-phase fraction 3.8, 95% CI 0.7-21.0; and 3pAI 3.8, 95% CI 0.7-21.0).

Discussion

Allelic imbalance on chromosome 3p was observed in 34% of the tumours examined in this study. Our observations are in accordance with findings of other researchers (6,32,33). Two of these groups (32,33) studied LOH at the 3p21-25 site, while Sato *et al* (6) found a high frequency of LOH at the p13-14.3 region on chromosome 3. Our results show AI in both regions. Several candidate suppressor genes on the 3p region have been suggested e.g. the von Hippel-Lindau gene at 3p25 (34), the acylpeptide hydrolase gene at 3p21.3 (35) and the gene which encodes for protein tyrosine phosphatase at 3p14 (36). These genes are possible candidate tumour suppressor genes for several cancer types, including breast cancer.

The microsatellite markers used are highly informative and well distributed along chromosome 3p allowing

Table III. Allelic imbalance (AI) on 3p in tumour DNA compared with other categorized prognostic factors by chi-square analysis.

Variable	AI/Total	%	p-value
All	48/140	34	
Node status			
negative	23/77	30	0.2237
positive	25/63	40	
Other metastases			
negative	43/131	33	0.4729
positive	2/4	50	
no information	3/5		
Tumour size			
< 2 cm	19/57	33	0.8802
> 2cm	28/81	35	
no information	1/2		
Histological type			
ductal	41/119	34	0.4514
lobular	4/16	25	
no information	3/5		
ER receptors			
fmol/mg protein			
≥ 10	30/107	28	0.0056
< 10	17/31	55	
no information	1/2		
PgR receptors			
fmol/mg protein			
≥ 25	25/89	28	0.0369
< 25	22/48	45	
no information	1/3		
Ploidy			
diploid	12/56	21	0.0050
non-diploid	35/78	45	
no information	1/6		
S-phase			
< 7%	11/63	17	0.0000
> 7%	35/66	53	
no information	2/11		
Age			
<50	18/50	36	0.7501
>50	30/90	33	

construction of deletion maps with fewer samples and less material than required when using RFLP markers. CA repeats will be useful for systematic screening of clinical samples since only a small amount of tissue is needed for PCR. A lower percentage of AI was detected with the D3S1076 marker at 3p21.1 compared with the higher number of abnormalities detected with the markers at 3p21.3-p22 and 3p14 suggesting that tumour suppressor genes could be located in these two chromosome regions.

Table IV. Multivariate analysis (proportional-hazard (Cox) regression) of survival in patients with breast cancer.

Factor	All patients (n=125)		
	Univariate p-value	Multivariate p-value	RR (with 95% CI)
Tumour size	0.0000	0.0010	8.28 (2.34-29.28)
Axillary nodal involvement	0.0000	0.0002	6.04 (2.35-15.53)
3p allelic imbalance	0.0000	0.0028	3.58 (1.55-8.26)
ER	0.0013	0.0394	2.46 (1.04-5.78)
S-phase fraction	0.0004	0.1560	1.87 (0.79-4.44)

Factors were categorized as shown in Table III. According to this model node status, tumour size, 3p allelic imbalance and ER content are significant predictors of hazard. RR = relative hazard of dying.

Microsatellite instability was observed in 4% of the cases studied, which supports the findings of Han *et al* (37) and Wooster *et al* (38) where 4% and 11%, respectively, of breast tumours exhibited replication errors (RER). The extra bands shown in Fig. 2 are similar to those observed in colorectal cancer (39,40), gastric (41), pancreatic cancer (37) and endometrial cancer (42). These bands are considered to be shifts in the electrophoretic mobility of dinucleotide repeat fragments caused by replication errors during tumour development. Mutations in the genes coding for DNA mismatch repair factors, hMSH2 and hMLH1, located on chromosomes 2p16 and 3p21.3, respectively, have been described in hereditary non-polyposis colorectal cancer (43-45). Thibodeau *et al* (46) suggest that in colorectal cancer the tumours may contain mutations in genes that when defective promote genetic instability at various loci, alterations which seem to be secondary events. All 6 cases exhibiting microsatellite instability are alive at the end of the study period. These observations could indicate a slower malignant progression for breast cancer patients showing RER phenotypes in spite of high S-phase status and 3pAI (Table II). 83% of the RER⁺ tumours show 3p AI which is of interest with respect to the location of the hMLH1 gene. Further studies are necessary to determine the importance of microsatellite instability in breast cancer and whether the loss of the hMLH1 gene in the tumours with 3p deletions could be responsible for the RER phenotypes.

We found no significant correlation between 3pAI and conventional prognostic factors such as patient age and the factors included in the TNM staging system i.e. tumour size, degree of axillary nodal involvement and whether distant metastases were recorded at diagnosis of the disease. However, 3pAI was significantly related to all the tumour biological variables that were investigated in the present series of patients (ER, PgR and S-phase). In the multivariate analyses 3pAI was invariably included as an independent prognostic variable. Our findings may be interpreted as further support for the claims that we need to include biological tumour behaviour in the prognostic evaluation of breast cancer patients (47,48). It has also been claimed in this context that the variables included in the TNM system reflect chronology and not tumour biology (47).

AI at 3p was found to be correlated with DNA non-diploidy, suggesting an association with an aberrant and instable genome. This supports the random origin of some deletions in the tumours. However, the high frequency and pattern of allelic imbalance and correlation with other biological factors of the tumour suggests the presence of a specific growth selection. The striking correlation between 3pAI and high S-phase fraction suggest that the putative suppressor gene(s) on 3p is linked to cell proliferation. Nevertheless, both factors were invariably included as independent prognostic factors in the multivariate analyses.

It has been shown in the node-negative setting that tumour size, PgR content and S-phase fraction are independent prognostic factors and a prognostic model that included these factors was capable of giving profound prognostic information (49). In the present study steroid receptor content was not an independent prognostic factor in the node-negative setting. However, 3pAI was included as an independent prognostic factor with tumour size and S-phase fraction.

The results of this study show that 3p abnormalities are common in breast cancer and have significant correlation with several clinicopathological factors. Moreover, our results show that 3pAI has independent prognostic value. Patients with tumours with 3pAI have a three to four-fold increase in relative risk of death both in the patient series as a whole as well as in the node-negative setting. We suggest that 3pAI can provide extra information about tumour biological behaviour and that screening for 3pAI may provide independent prognostic information which should be further investigated.

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References

1. Steeg PS: Suppressor genes in breast cancer: an overview. *Cancer Treat Res* 61: 45-57, 1992.
2. Chen LC, Kurisu W, Ljung BM, Goldman ES, Moore D II, Smith HS: Heterogeneity for allelic loss in human breast cancer. *J Natl Cancer Inst* 84: 506-510, 1992.
3. Killary AM, Wolf ME, Giambernardi TA and Naylor S: Definition of a tumor suppressor locus within human chromosome 3p21-p22. *Proc Natl Acad Sci USA* 89: 10877-10881, 1992.
4. Shimizu M, Yokota J, Mori N, Shuin T, Shinoda M, Terada M and Oshimura M: Introduction of normal chromosome 3p modulates the tumorigenicity of a human renal cell carcinoma cell line YCR. *Oncogene* 5: 185-194, 1990.
5. Imreh S, Kholodnyuk I, Allikmetts R, Stanbridge EJ, Zabarovsky ER and Klein G: Nonrandom loss of human chromosome 3 fragments from mouse-human microcell hybrids following progressive growth in SCID mice. *Genes Chromosom Cancer* (In press).
6. Sato T, Akiyama F, Sakamoto G, Kasumi F and Nakamura Y: Accumulation of genetic alterations and progression of primary breast cancer. *Cancer Res* 51: 5794-5799, 1991.
7. Zbar B, Brauch H, Talmadge C, Linehan M: Loss of alleles of loci on the short arm of chromosome 3 in renal cell carcinoma. *Nature* 327: 721-724, 1987.
8. Minna JD: The molecular biology of lung cancer pathogenesis. *Chest* 103 (suppl 4): 449S-456S, 1993.
9. Ehlen T and Dubeau L: Loss of heterozygosity on chromosomal segments 3p, 6q and 11p in human ovarian carcinomas. *Oncogene* 5: 219-223, 1990.
10. Yokota J, Tsukada Y, Nakajima T, Gotch M, Shimosato Y, Mori N, Tsunokawa Y, Sugimura T, Terada M: Loss of heterozygosity on the short arm of chromosome 3 in carcinoma of the uterine cervix. *Cancer Res* 49: 3598-3601, 1989.
11. Lothe RA, Fosså SD, Stenwig AE, Nakamura Y, White R, Børresen AL, Brøgger A: Loss of 3p or 11p alleles is associated with testicular cancer tumors. *Genomics* 5: 134-138, 1989.
12. Lo KW, Tsao SW, Leung SF, Choi PHK, Lee JCK and Huang DP: Detailed deletion mapping on the short arm of chromosome 3 in nasopharyngeal carcinoma. *Int J Oncol* 4: 1359-1364, 1994.
13. Pandis N, Jin Y, Limon J, Bardi G, Idvall I, Mandahl N, Mitelman F and Heim S: Interstitial deletion of the short arm of chromosome 3 as a primary chromosome abnormality in carcinomas of the breast. *Genes Chromosom Cancer* 6: 151-155, 1993.
14. Gruis NA, Abeln ECA, Bardoel AFJ, Devilee P, Frants RR and Cornelisse CJ: PCR-based microsatellite polymorphisms in the detection of loss of heterozygosity in fresh and archival tumour tissue. *Br J Cancer* 68: 308-313, 1993.
15. Miller SA, Dykes DD and Polesky HF: A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 16: 1215, 1988.
16. Maniatis T, Fritsch EF and Sambrook J: *Molecular Cloning: A laboratory Manual*. Cold Spring Harbor Laboratory Press, New York, 1982.
17. Thomthwaite JT, Sugerbaker EV and Temple WJ: Preparation of tissues for DNA flow cytometric analysis. *Cytometry* 1: 229-237, 1980.
18. Hedley DW, Friedlander ML and Taylor IW: Application of DNA flow cytometry to paraffin-embedded archival material for the study of aneuploidy and its clinical significance. *Cytometry* 6: 327-333, 1985.
19. Hiddeman W, Schuman J and Andreeff M: Convention of nomenclature for DNA cytometry. *Cytometry* 5: 445-446, 1984.
20. Baish H, Gohde W and Linden WA: Analysis of PCP-data to determine the fraction of cells in various phases of cell cycle. *Rad Environm Biophys* 12: 31-9, 1975.
21. Kaplan EL and Meier P: Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 53: 457-481, 1958.
22. Mantel N: Evaluation of survival data and two new rank order statistics arising in its consideration. *Cancer Chemother Rep* 50: 163-170, 1966.
23. Cox DR: Regression models and life-tables. *J R Stat Soc (B)* 34: 187-220, 1972.
24. Latif F, Glenn GM, Brauch H, Delisio J, Hampsch K, Orcutt ML, Zbar B and Lerman MI: A new polymorphic probe on chromosome 3p: LIB31-38 (D3S726). *Nucleic Acids Res* 19: 962, 1991.
25. Hudson TJ, Engelstein M, Lee MK, Ho EC, Rubenfield MJ, Adams CP, Housman DE and Dracopoli NC: Isolation and chromosomal assignment of 100 highly informative human simple sequence repeat polymorphisms. *Genomics* 13: 622-629, 1992.
26. Erlandsson R, Bergerheim USR, Boldog F, Marcsek Z, Kunimi K, Lin BYT, Ingvarsson S, Castresana JS, Lee WH, Lee E, Klein G and Sümegi J: A gene near the D3F15S2 site on 3p is expressed in normal human kidney but not or only at a severely reduced level in 11 of 15 primary renal cell carcinomas (RCC). *Oncogene* 5: 1207-1211, 1990.
27. Carrith B, Welch HM and Parry-Jones NJ: Sequences homologous to the human D1S1 locus present on human chromosome 3. *Am J Hum Genet* 38: 428-436, 1986.
28. Jones MH, Yamakawa K and Nakamura Y: Isolation and characterization of 19 dinucleotide repeat polymorphisms on chromosome 3p. *Human Mol Genet* 1: 131-133, 1992.
29. O'Connell P, Rains D, Wilkie P, Garcia D, Matsunami N, Albertsen H, Weber J, Weissenbach J, White R and Naylor S: A microsatellite genetic map for chromosome 3. *Cytogenet Cell Genet* (In press).
30. Naylor SL, Garcia DK, Schantz LJ, Huang W, Ghosh-Choudhury N, Chinn RB, Wolf ME and Killary AM: Ordering of loci on human chromosome 3 using microcell and radiation hybrids. *Cytogenet Cell Genet* 58: 1879, 1991.
31. Granqvist M, Xiang K, Seino M, Fukumoto H and Bell GI: Dinucleotide repeat polymorphism in human GLUT2/liver facilitative glucose transporter gene on chromosome 3. *Nucleic Acids Res* 19: 4791, 1991.
32. Ali IU, Lidereau R and Callahan R: Presence of two members of *c-erbA* receptor gene family (*c-erbA* and *c-erbA2*) in smallest region of somatic homozygosity on chromosome 3p21-p25 in human breast carcinoma. *J Natl Cancer Inst* 81: 1815-1820, 1989.
33. Devilee P, van den Broek M, Kuipers-Dijkshoorn N, Kolluri R, Khan PM, Pearson PL and Cornelisse CJ: At least four different chromosomal regions are involved in loss of heterozygosity in human breast carcinoma. *Genomics* 5: 554-560, 1989.
34. Latif F, Tory K, Gnarr J, Yao M, Duh F, Orcutt ML, Stackhouse T, Kuzmin I, Modi W, Geil L, Schmidt L, Zhou F, Li H, Wei MH, Chen F, Glenn G, Choyke P, Walther MM, Weng Y, Duan DR, Dean M, Glavac D, Richards FM, Crossey PA, Ferguson-Smith MA, Le Paslier D, Chumakov I, Cohen D, Chinnault AC, Maher ER, Linehan WM, Zbar B and Lerman MI: Identification of the von Hippel-Lindau disease tumor suppressor gene. *Science* 260: 1317-1320, 1993.
35. Erlandsson R, Boldog F, Persson B, Zabarovsky ER, Allikmetts RL, Sümegi J, Klein G and Jörnvall H: The gene from the short arm of chromosome 3, at D3F15S2, frequently deleted in renal cell carcinoma, encodes acylpeptide hydrolase. *Oncogene* 6: 1293-1295, 1991.
36. LaForgia S, Morse B, Levy J, Barnea G, Cannizzaro LA, Li F, Nowell PC, Boghosian-Sell L, Glick J, Weston A, Harris CC, Drabkin H, Patterson D, Croce CM, Schlessinger J and Huebner K: Receptor protein-tyrosine phosphatase is a candidate tumor suppressor gene at human chromosome region 3p21. *Proc Natl Acad Sci USA* 88: 5036-5040, 1991.
37. Han H, Yanagisawa A, Kato Y, Park J and Nakamura Y: Genetic instability in pancreatic cancer and poorly differentiated type of gastric cancer. *Cancer Res* 53: 5087-5089, 1993.
38. Wooster R, Cleton-Jansen AM, Collins N, Mangion J, Cornelis RS, Cooper CS, Gusterson BA, Ponder BAJ, von Deimling A, Wiestler OD, Cornelisse CJ, Devilee P and Stratton MR: Instability of short tandem repeats (microsatellites) in human cancers. *Nature Genet* 6: 152-156, 1994.
39. Aaltonen LA, Peltomäki P, Leach FS, Sistonen P, Pylkkänen L, Mecklin J, Järvinen H, Powell SM, Jen J, Hamilton SR, Petersen GM, Kinzler KW, Vogelstein B and de la Chapelle A: Clues to the pathogenesis of familial colorectal cancer. *Science* 260: 812-816, 1993.

40. Peltomäki P, Lothe RA, Aaltonen LA, Pylkkänen L, Nyström-Lahti M, Seruca R, David L, Holm R, Ryberg D, Haugen A, Brøgger A, Børresen AL and de la Chapelle A: Microsatellite instability is associated with tumors that characterize the hereditary non-polyposis colorectal carcinoma syndrome. *Cancer Res* 53: 5853-5855, 1993.
41. Mironov NM, Aguelon MA, Potapova GI, Omori Y, Gorbunov OL, Klimenkov AA and Yamasaki H: Alterations of (CA)_n DNA repeats and tumor suppressor genes in human gastric cancer. *Cancer Res* 54: 41-44, 1993.
42. Risinger JI, Berchuck A, Kohler MF, Watson P, Lynch HT and Boyd J: Genetic instability of microsatellites in endometrial carcinoma. *Cancer Res* 53: 5100-5103, 1993.
43. Papadopoulos N, Nicolaides NC, Wei Y, Ruben SM, Carter KC, Rosen CA, Haseltine WA, Fleischmann RD, Fraser CM, Adams MD, Venter JC, Hamilton SR, Petersen GM, Watson P, Lynch HT, Peltomäki P, Mecklin JP, de la Chapelle A, Kinzler KW and Vogelstein B: Mutation of a mutL homolog in hereditary colon cancer. *Science* 263: 1625-1629, 1994.
44. Bronner CE, Baker SM, Morrison PT, Warren G, Smith LG, Lescoe MK, Kane M, Earabino C, Lipford J, Lindblom A, Tannergard P, Bollag RJ, Godwin AR, Ward DC, Nordenskjöld M, Fishel R, Kolodner R and Liskay RM: Mutation in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary non-polyposis colon cancer. *Nature* 368: 258-261, 1994.
45. Leach FS, Nicolaides NC, Papadopoulos N, Liu B, Jen J, Parsons R, Peltomäki P, Sistonen P, Aaltonen LA, Nyström-Lahti M, Guan XY, Zhang J, Meltzer PS, Yu J, Kao F, Chen DJ, Cerosaietti KM, Fournier REK, Todd S, Lewis T, Leach RJ, Naylor SL, Weissenbach J, Mecklin J, Järvinen H, Petersen GM, Hamilton SR, Green J, Jass J, Watson P, Lynch HT, Trent JM, de la Chapelle A, Kinzler KW and Vogelstein B: Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. *Cell* 75: 1215-1225, 1993.
46. Thibodeau SN, Bren G and Schaid D: Microsatellite instability in cancer of the proximal colon. *Science* 260: 816-819, 1993.
47. Mittra L, MacRae K: A meta-analysis of reported correlations between prognostic factors in breast cancer: does axillary lymph node metastasis represent biology or chronology? *Eur J Cancer* 27: 1574-1583, 1991.
48. Barr L, Baum M: Time to abandon TNM staging of breast cancer. *Lancet* 339: 915-917, 1992.
49. Sigurdsson H, Baldertorp B, Borg A, Dalberg M, Fernö M, Killander D and Olsson H: Indicators of prognosis in node-negative breast cancer. *N Engl J Med* 322: 1045-1053, 1990.